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Sensitivity of Juvenile Atlantic Sturgeon to Three Therapeutic Chemicals Used in Aquaculture

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Abstract.—Static tests were performed to determine the acute toxicities of formalin, chloramine-T, and sodium chloride on juvenile Atlantic sturgeon *Acipenser oxyrinchus* (average weight, 2.1 g) in oxygen-saturated dilution water (total hardness, 86.5 mg/L) at 17°C. The concentrations that were lethal to 50% of the test fish over a 96-h period (the 96-h LC50 values) were 31.00 μL/L for formalin, 7.73 mg/L for chloramine-T, and 9.735 g/L for sodium chloride. These findings indicate that the therapeutic concentrations and exposure times that are commonly employed in aquaculture would not be acutely toxic to Atlantic sturgeon.

Therapeutic chemicals are routinely used to treat fish diseases in aquaculture, particularly those caused by parasites. A considerable amount of information on disease control using chemical treatments has been published (Fish 1940; Leteux and Meyer 1972; Piper et al. 1982; Bills et al.1993). However, the recommendations in the literature have not always been effective under local environmental conditions (i.e., the physical and chemical conditions of the water) and for species new to aquaculture. Although a variety of chemicals are available to manage disease and parasites, their use may pose problems such as toxicity to fish and the emergence of drug-resistant pathogens (Post 1987). As tolerance to chemicals may vary amongspecies, the relative sensitivity of individual species should be determined before recommending specific chemical treatments.

Current interest in the culture and restoration of Atlantic sturgeon *Acipenser oxyrinchus* has prompted federal and state agencies to place more emphasis on the development of rearing techniques for all life stages of the species. Intensive culture of Atlantic sturgeon requires handling, which often results in stress (Barton et al. 2000), injury, and increased susceptibility to disease (LaPatra et al. 1996) and parasites. Mohler et al. (2000) provided baseline data concerning the application of formalin to first-feeding Atlantic sturgeon fry. They reported that a 1-h static formalin treatment at 150 mg/L was successful in controlling mortality caused by protozoa in the genus *Chilodonella*.

The primary goal of this study was to determine the concentrations of formalin, chloramine-T, and sodium chloride that are lethal to 50% of Atlantic sturgeon fingerlings exposed to those chemicalsfor 96 h (i.e., the 96-h LC50 values). These values will provide fishery managers and researchers with baseline guidance in the use of these chemicals for Atlantic sturgeon aquaculture.

Methods

Test fish.—Tests were conducted using 1998 year-class Atlantic sturgeon fingerlings produced from eggs of Hudson River origin. Eggs were incubated and fingerlings reared at the Northeast Fishery Center (NEFC), Lamar, Pennsylvania, in spring water (Mohler et al. 1996). Fish were maintained according to standard procedures for handling fish for bioassay (ASTM 1997). Feed was withheld and fish were acclimated to the desired water chemistry and temperature for at least 48 h before testing.

Test chemicals.—The chemicals and their suppliers were as follows: reagent-grade formalin (37% formaldehyde, 12% methanol inhibited; Argent Chemical Company, Redmond, Washington), reagent-grade chloramine-T (12% chloride; Akzo Nobel Chemicals, Inc., Dobbs Ferry, New York), and untreated sodium chloride (Cargill, Inc., Minneapolis, Minnesota).

Test solutions were analyzed according to procedures described by the U.S. Environmental Protection Agency (EPA 1986) to confirm nominal concentrations. Samples were withdrawn from the control, high, medium, and low exposures at the beginning of each definitive test.

Dilution water.—Water used in preparation of the test solutions was NEFC spring water. Total water hardness (74–99 mg/L as $CaCO_3$), total alkalinity (69–95 mg/L as $CaCO_3$), and specific conductivity (197–215 μS/cm) were measured prior to each test according to procedures in EPA (1986). The pH of dilution water was measured at

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TABLE 1.—Mortality of juvenile Atlantic sturgeon in the chloramine-T, formalin, and sodium chloride toxicity tests. Data are pooled from three replicate tests; LD50 is the concentration that has lethal to 50% of the fish in a given group, and CI stands for confidence interval.

Concentration	Total number of fish (N)	Number of organisms dead at 3 h	6 h	12 h	24 h	48 h	72 h	96 h	LC50 and 95% CI
Chloramine-T (mg/L):									7.73 (6.7–11.2)
0.00	21	0	0	0	0	0	0	1	
4.00	21	0	0	0	0	0	0	0	
6.70	21	0	0	0	0	0	1	5	
11.20	21	0	0	0	0	20	21	21	
18.60	21	0	0	0	12	21	21	21	
31.00	21	0	0	0	21	21	21	21	
Formalin (μL/L):									31.00 (27.3–35.2)
0.00	21	0	0	0	0	0	0	0	
19.40	21	0	0	0	0	0	0	1	
32.40	21	0	0	0	0	0	11	11	
54.00	21	0	0	0	0	0	21	21	
90.00	21	0	0	0	0	21	21	21	
150.00	21	0	0	0	21	21	21	21	
Sodium chloride (g/L):									9.73 (8.67–10.82)
0.00	21	0	0	0	0	0	0	0	
4.28	21	0	0	0	0	0	0	0	
7.13	21	0	0	0	0	0	0	1	
11.88	21	0	0	0	0	5	15	18	
19.80	21	0	1	6	21	21	21	21	
33.00	21	18	21	21	21	21	21	21	

the beginning and end of each test in the control, high, medium, and low concentrations of test material. The pH ranged from 7.5 to 8.4. There were no chemical or concentration effects on pH. Temperatures were maintained at 17°C by holding the test jars in a constant-temperature water bath.

Dissolved oxygen concentrations were measured at the beginning of the experiment and at least every 48 h thereafter until the tests were terminated. These concentrations met the criteria recommended by ASTM (1997) at 0 h (saturation = 78–100%) and 96 h (saturation = 40–75%) across all chemical tests. At 48 h, the dissolved oxygen concentrations for formalin (52–56% saturation) in the medium test concentrations were below the recommended criteria (60% saturation); those for sodium chloride (40–53% saturation) were also below the recommended criteria at 48 h. Possible reasons for this include partial mortality at those concentrations or the increased gill activity of stressed fish. However, because the tests met the dissolved oxygen criteria at 96 h of exposure, we believe that the tests were not compromised and that the results are valid.

Acute toxicity tests.—Static acute toxicity tests were conducted according to procedures in EPA (1975) and ASTM (1997). Juvenile Atlantic sturgeon were exposed to selected concentrations of formalin, chloramine-T, and sodium chloride in 19.6-L glass jars containing 18 L of oxygen-saturated water. Exposure concentrations were determined from a range-finding test that consisted of a 48-h static exposure of test fish to a control and five concentrations.

Each definitive test was performed in triplicate and consisted of a 0.6 dilution series with five exposure concentrations and a control (Table 1) randomly distributed among the test chambers by adding one fish at a time within 30 min after test material was added. This process was repeated until each chamber contained seven fish (average weight, 2.1 g; average total length, 81 mm; average loading density, 0.88 g/L). Test fish were not fed during the 96-h test period. Mortality was recorded at 3, 6, 12, 24, 48, 72, and 96 h after test initiation. Dead fish were removed at each observation time. Tests were conducted under ambient lighting supplemented by overhead fluorescent lighting from 0800 to 1600 hours.

Statistical analysis.—The LC50 concentrations and their 95% confidence limits were estimated for formalin and sodium chloride by means of EPA probit analysis. A nonlinear interpolative procedure (Stephan 1997) was used to estimate the LC50 for chloramine-T when the requirements were not met for probit analysis (i.e., when there were data with two or more concentrations at which the percent dead is between 0 and 100) according to procedures in ASTM (1997).

Results and Discussion

Chloramine-T, formalin, and sodium chloride exerted measurable lethal effects on juvenile Atlantic sturgeon. The 96-h LC50 for chloramine-T was 7.73 mg/L (Figure 1A). Chloramine-T has been tested for acute lethal toxicity in a number of fishes. Bills et al. (1988, 1993) reported 96-h LC50s of 2.80 mg/L, 3.73 mg/L, and 9.70 mg/L for rainbow trout *Oncorhynchus mykiss*, channel catfish *Ictalurus punctatus*, and striped bass *Morone saxatilis*, respectively, under water quality conditions that were similar to those in our study (i.e., well-oxygenated, slightly alkaline water with medium hardness). Our data indicate that Atlantic sturgeon are more tolerant than rainbow trout and channel catfish and are similar to or slightly less resistant than striped bass in their sensitivity to chloramine-T under these conditions.

Currently, chloramine-T is being used under Investigational New Animal Drug exemption 4000, granted by the U.S. Food and Drug Administration (FDA) for investigational purposes. Dawson and Davis (1997) recommended using 10 mg/L of chloramine-T in a 1-h bath treatment for the prevention and treatment of infectious gill and skin diseases in salmonids. For rainbow trout, From (1980) recommended using 6.5 mg/L in 1-h treatments with flow-through water and 4.5 mg/L in ponds. Our data show that juvenile Atlantic sturgeon can tolerate up to 31 mg/L for 3 h (Table 1). Other studies indicate that the toxicity of chloramine-T is affected by variations in water chemistry, especially pH (Cross and Hursey 1973; Bills et al. 1988). It is likely that chloramine-T would be more toxic to Atlantic sturgeon in acidic water, as this has been shown for other species (Bills et al. 1988, 1993).

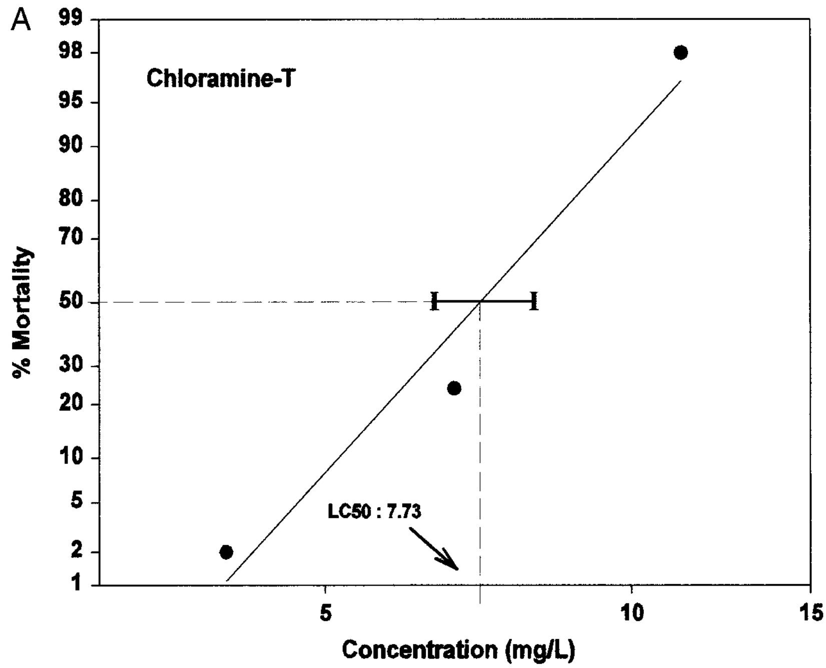
In our study, the 96-h LC50 for formalin was 31 μL/L (Figure 1B) for juvenile Atlantic sturgeon. This concentration is similar to the 96-h LC50s reported for striped bass, which range from 30 to 56 μL/L for varying water temperatures (Bills et al. 1993). A wide range of tolerances for formalin exists amongdifferent fish species. Bills et al. (1977) reported 96-h LC50s ranging from 62 μL/L for black bullheads *Ameiurus melas* to 173 μL/L for green sunfish *Lepomis cyanellus* and Atlantic salmon *Salmo salar* under varying conditions of water temperature, hardness, and pH. These results demonstrated that water hardness had no effect on toxicity. In soft water, formalin was more toxic to channel catfish and rainbow trout at a pH of 9.5 than at lower pHs. Additionally, formalin was more toxic to channel catfish and rainbow trout in warm water (≥17°C) than in cold water (<17°C). Similar results were found for striped bass (Bills et al. 1993). Because the toxicity of formalin is affected by variations in water chemistry and temperature, we expected that formalin would be less toxic to Atlantic sturgeon exposed in hard water at lower temperatures. Our data indicate that Atlantic sturgeon are similar to striped bass in their sensitivity to formalin and are about four times more sensitive than salmonids.

Formalin is currently approved by the FDA as a new animal drug for use on all finfish as a parasiticide and fungicide at concentrations of up to 250 mg/L for 1 h in tanks and raceways and 15–25 mg/L in ponds (FDA 1998). Our data indicate that juvenile Atlantic sturgeon can tolerate up to 150 mg/L for 3 h with no mortality under specified conditions (Table 1).

The 96-h LC50 value for sodium chloride was 9.73 g/L (Figure 1C) in treatment water of medium hardness. This value is higher than the 96-h LC50 reported by Hughes (1969) for striped bass juveniles tested in soft water (5.0 g/L). However, Grizzle and Mauldin (1995) determined that the acute toxicity effects of 5.0 g/L to striped bass juveniles can be eliminated when Ca^{2+} ions are added to soft water. The minimum amount of Ca^{2+} ions required to prevent the toxic effects of sodium chloride to Atlantic sturgeon needs to be determined and should be considered in the application of sodium chloride as a chemical treatment.

Salinity tolerance (abrupt transfer into saline water) has been determined for several species of fish. Laboratory studies by Allen and Avault (1970) indicate that fingerling-size channel catfish have a salinity tolerance of 12 g/L. This approaches the lower value of the tolerance range (15–25 g/L) recorded by Wagner et al. (1969) for 2.9-g chinook salmon *Oncorhynchus tshawytscha*. McEnroe and Cech (1985) reported a gradual increase in the upper salinity tolerance (abrupt transfer) of juvenile white sturgeon *Acipenser transmontanus* with increasing fish weight (i.e., 5–10 g/L for 0.4–0.9-g fish, 10–15 g/L for 0.7–1.8-g fish, and 15 g/L for 4.9–50.0-g fish). Increasing salinity tolerance with size has also been reported in other sturgeon species (Gershanovich et al. 1991; Kirschner 1995; Altinok et al. 1998) and should be considered in the application of sodium chloride as a therapeutant.

Salt has been routinely used in aquaculture to stimulate mucus flow in fish, help expose parasites and bacteria to subsequent chemical treatments,



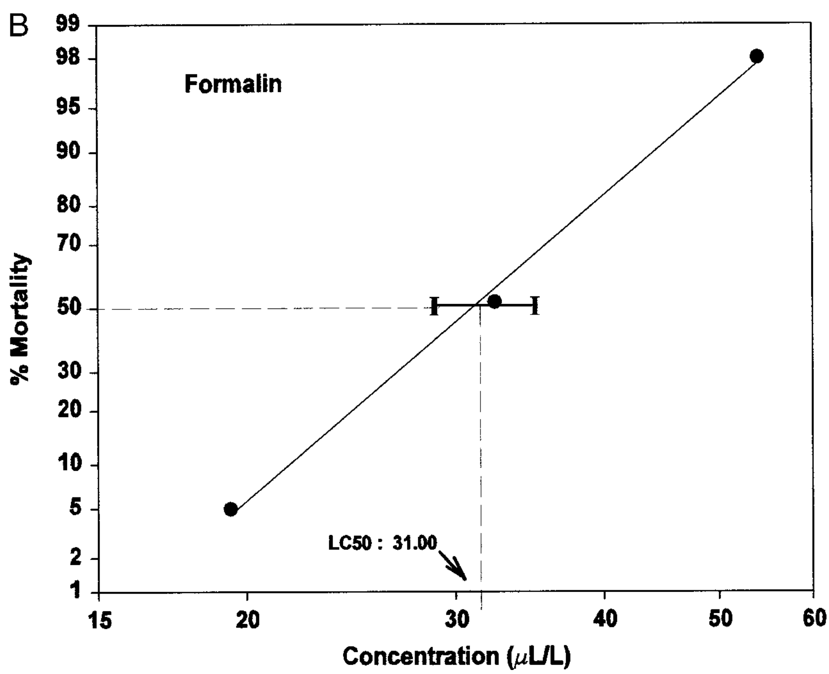


FIGURE 1.—The estimated 96-h LC50 of **(A)** chloramine-T, **(B)** formalin, and **(C)** sodium chloride for juvenile Atlantic sturgeon.

and as a therapeutic to reduce osmoregulatory stress. Currently, sodium chloride is listed as an unapproved drug of low regulatory priority for FDA. The FDA recommends using 30 g/L in a static treatment for 10–30 min when sodium chloride is used as a parasiticide and from 5–10 g/L for an indefinite period when it is used as a osmoregulatory aid (Schnick et al. 1989; Texas Ag-

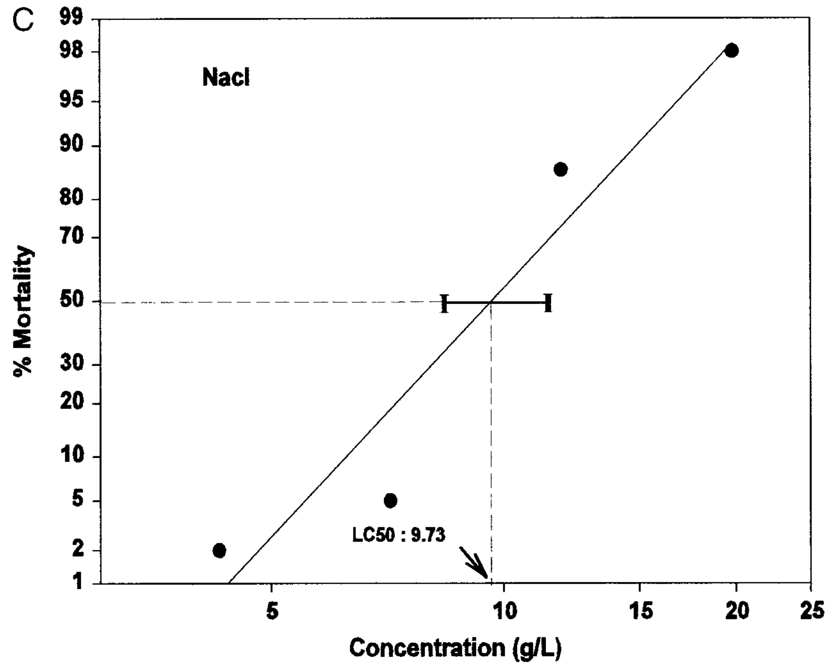


FIGURE 1.—Continued.

ricultural Extension Service 1994). Our data indicate that juvenile Atlantic sturgeon can tolerate 19.8 g/L for up to 3 h with no mortality (Table 1).

Summary

Our study shows that Atlantic sturgeon juveniles can tolerate relatively high concentrations of chloramine-T and formalin for short periods (up to 12 h) but are highly sensitive to low concentrations over longer periods. In contrast, these fish are highly sensitive to short exposures of relatively high concentrations of sodium chloride but appear to tolerate lower concentrations.

Under our test conditions, juvenile Atlantic sturgeon appear to be similar to striped bass in their sensitivity to the chemical treatments. Dwyer et al. (2000) found that the sensitivity of Atlantic sturgeon was similar to that of rainbow trout when exposed to various classes of contaminants. Water characteristics such as pH, hardness, and temperature may modify the response of fish to a treatment chemical, and culturists and researchers should conduct sensitivity tests on fish at their own facilities before chemical applications are made.

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